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(71) Applicant *(for all designated States except US)*: **GLAXO-SMITHKLINE** [GB/GB]: Corporate Intellectual Property, Two New Horizons court, Brentford, Middlesex TW8 9EP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LEWIS, Alan, Peter** [GB/GB]; Glaxo Wellcome plc, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB). **RAY, Keith, Paul** [GB/GB]; Glaxo Wellcome plc, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB).

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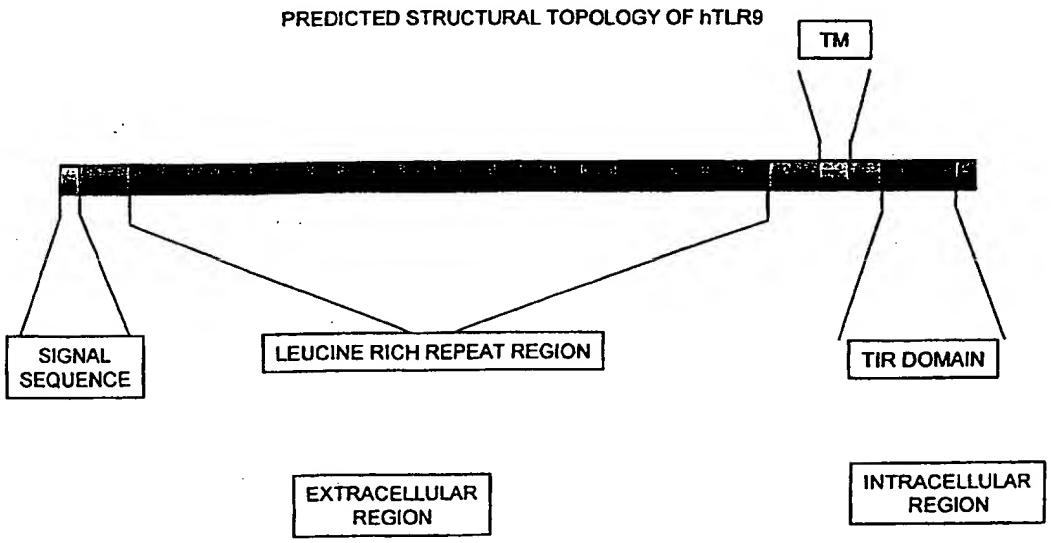
(54) Title: TOLL-LIKE RECEPTOR



(57) Abstract: An isolated Toll-like-receptor polypeptide comprises the amino acid sequence of SEQ ID NO: 2, a variant or a fragment thereof which has immunomodulatory activity. Polynucleotides encoding such a Toll-like receptor are also described. A method for identification of a substance that modulates Toll-like receptor activity comprises contacting a polypeptide of the invention with a test substance and monitoring for immunomodulatory activity.

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Toll-like Receptor

Field of the Invention

The present invention relates to a novel Toll-like receptor or a variant thereof. A variant may demonstrate Toll-like receptor activity such as activation of NFκB, or may inhibit Toll-like receptor activity.

Background of the Invention

A family of human Toll-like receptors has been described in the literature. These receptors are termed Toll-like receptors in view of common structural features shared with a Drosophila Toll (dToll) receptor molecule which is involved in embryonic development. Toll and Toll-Like receptors are type I transmembrane proteins, with extracellular leucine rich repeat motifs and an intracellular signalling domain homologous to that of members of the interleukin 1 receptor superfamily.

Drosophila Toll also plays an important role in the adult fly and is involved in immune surveillance mechanisms required for recognition of bacterial and fungal pathogens and regulation of specific innate immune defence gene expression. Activation of dToll receptors in response to infection by specific micro-organisms is thought to require the production of a protein ligand called Spaetzle. The human Toll-like receptors (hTLRs) are also thought to participate in mechanisms of innate immunity and inflammation acting as pattern recognition receptors (PRRs) for bacteria and other micro-organisms. hTLRs are expressed on antigen presenting cells including monocytes and dendritic cells and like dToll play roles in innate immunity. TLRs can elicit pro-inflammatory cytokine production and induce expression of cell surface co-stimulatory receptors required for activation of T-cells. Some hTLRs may help to co-ordinate interactions between cells of the innate and acquired immune systems to orchestrate an integrated immune response to infection.

The specific functions of two mammalian TLRs, TLR2 and TLR4, have recently been identified. TLR2 and TLR4 are involved in mediating host responses to gram positive and gram negative bacteria through recognition of specific bacterial wall components. It has also recently been shown that TLR4 mediates responses to certain viral proteins including respiratory syncytial virus (RSV) (Nature Immunology 1: 398 2000).

Additionally TLRs may form heterodimeric functional complexes. Components of the intracellular signal transduction pathways of some hTLRs appear to be shared with interleukin-1 (IL-1) receptor transduction pathways. Stimulation of TLR2 and TLR4 leads to activation of NFκB via an adapter protein MyD88 and recruitment of the IL-1 receptor associated kinases (IRAKs).

Summary of the Invention

A novel Toll-like receptor is now provided which is a screening target for the identification and development of novel pharmaceutical agents which modulate the activity of the receptor and in particular have immunomodulatory activity. These agents may be used in the treatment and/or prophylaxis of inflammatory diseases, cardiovascular diseases, systemic infections and autoimmune diseases, such as asthma, rhinitis, chronic obstructive pulmonary disease (COPD), emphysema, inflammatory bowel disease such as ulcerative colitis and Crohn's disease, rheumatoid arthritis, osteoarthritis, psoriasis, Alzheimers disease, atherosclerosis, viral, fungal and bacterial infections, septic shock syndrome associated with systemic infection involving gram positive and gram negative bacteria, diabetes, Multiple Sclerosis. These agents may also be used as immunoadjuvants to enhance or alter the immune response in vaccine therapy.

Accordingly, the present invention provides an isolated Toll-like-receptor polypeptide which comprises:

(i) the amino acid sequence of SEQ ID NO: 2;

(ii) a variant of (i) which has immunomodulatory activity; or

(iii) a fragment of (i) or (ii) which retains immunomodulatory activity.

Preferably, a variant has at least 80% identity to the amino acid sequence of SEQ ID NO: 2, more preferably at least 95% identity therewith, for example 97% identity therewith.

The invention also provides a polynucleotide encoding a polypeptide of the invention. Such a polynucleotide may be a polynucleotide which encodes a Toll-like receptor polypeptide which has immunomodulatory activity. The polynucleotides of the invention may be DNA or RNA, for example mRNA. A polynucleotide according to the invention comprises:

(a) the nucleic acid sequence of SEQ ID NO: 1 and/or a sequence complementary thereto;

(b) a sequence which hybridises under stringent conditions to a sequence as defined in (a);

(c) a sequence that is degenerate as a result of the genetic code to a sequence as defined in (a) or (b); or

(d) a sequence having at least 60% percent identity to a sequence as defined in (a), (b) or (c).

The present invention also provides a polypeptide expressed from a polynucleotide according to (a), (b), (c) or (d) above, in particular a polypeptide comprising a toll-like receptor according to the invention, encoded by the mRNA derived from a DNA sequence according to (a) or (b) above, thus the invention provides an isolated toll-like receptor polypeptide which is obtainable by expression in vitro or in vivo of a DNA molecule comprising the sequence of nucleotides as shown in SEQ ID NO.1.

The polypeptides of SEQ ID NO 2 and SEQ ID NO 4, which are different isoforms expressed from the multiple exon tlr9 gene, are herein referred to as TLR9 and TLR9-A, respectively. TLR9-A is encoded by the nucleotide sequence of SEQ ID No. 3, which is encoded within SEQ ID No.1 except for the initiating methionine, that is encoded by a second exon as illustrated in figure 1. (see Hemmi et. al. Nature 408, 740-745 2000; Du et al, European Cytokine Network, 11: 362-371, 2000;Chuang and Ulevitch, European Cytokine Network, 11: 372-378 for isolation of the cDNAs, and corresponding sequence database accessions EMBL:AB045180, EMBL:AF259262, EMBL:AF245704).

In further aspects of the invention we provide:

- an expression vector capable of expressing a polypeptide of the invention comprising a polynucleotide as defined above.
- a host cell comprising an expression vector of the invention.
- an antibody specific for a polypeptide of the invention.
- a method for identification of a compound that modulates Toll-like receptor activity, comprising contacting a polypeptide of the invention with a test compound and monitoring for immunomodulatory activity.

Compounds which are identifiable in accordance with this method may be used in the treatment of a subject having a disorder that is responsive to Toll-like receptor modulation such as an inflammatory or cardiovascular disorder or systemic infection or autoimmune disease, including asthma, chronic obstructive pulmonary disease (COPD), emphysema, inflammatory bowel disease such as ulcerative colitis and Crohn's disease, rheumatoid arthritis, osteoarthritis, psoriasis, viral, fungal and bacterial infections, Alzheimers disease, atherosclerosis, septic shock syndrome associated with systemic infection involving gram positive and gram negative bacteria, diabetes and Multiple Sclerosis. In particular, compounds which are identifiable in accordance with this method may be used in the treatment of a subject having allergic asthma or

rhinitis. Further, such compounds may have immunomodulatory activity and be of use in the treatment of, or as adjuvants in vaccination against, bacterial or viral infections or as components of anti-cancer vaccines.

Compounds identifiable in accordance with this method include, in particular, synthetic or naturally occurring oligopeptides or polypeptides, oligonucleotides or polynucleotides which bind directly to the Toll-like receptor of the invention, and synthetic or naturally occurring oligopeptides or polypeptides, oligonucleotides or polynucleotides which modulate the Toll-like receptor of the present invention via one or more intermediate signal transducers. Such oligo- or polynucleotides may be "CG-rich" sequences or sequences including one or more unmethylated CpG nucleotide pairs.

In an alternative aspect of the invention, a polypeptide comprises a fragment or variant of SEQ ID NO 2 which is capable of inhibiting the activity of TLR9 or TLR9-A, for use in the treatment of an immune or inflammatory disorder.

In a further aspect of the invention, a polypeptide or polynucleotide in accordance with the invention or a compound identifiable in accordance with the invention is provided for use as an adjuvant or as an immunotherapeutic agent, for example in a vaccine.

Brief Description of the Sequences

SEQ ID NO: 1 is the amino acid sequence of human protein TLR9 and its encoding DNA;

SEQ ID NO: 2 is the amino acid sequence alone of TLR9;

SEQ ID NO: 3 is the amino acid sequence of human protein TLR9-A and its encoding cDNA (EMBL:AF259262);

SEQ ID NO: 4 is the amino acid sequence alone of TLR9-A (Hemmi et al.).

Brief Description of th Drawings

Fig. 1 is a diagrammatic illustration showing the exon arrangement encoding TLR9 and TLR9-A;

Fig. 2 shows tissue distribution data for TLR9 (using a human tissue cDNA plate). The profile shows predominant expression in tonsil and adenoid tissues with lower levels of expression detected in adipose, adrenal, foetal brain, cerebellum, jejunum, lung, myometrium, omentum, head of pancreas, rectum, skeletal muscle, spleen and thymus tissues;

Fig. 3 shows tissue distribution data for TLR9 (using a human disease cDNA plate). The profile shows predominant expression in lung tissue, bone marrow and PBMC with lower levels of expression detected in some colon, breast and brain/cerebellum samples;

Fig. 4 illustrates, in diagrammatic form, the predicted structural topology of human TLR9 - "TM" is the transmembrane portion, "TIR" is the cytosolic region conserved among interleukin and toll-like receptors known as the Toll Interleukin Receptor domain.

Detailed Description of the Invention

A single open reading frame was identified in genomic DNA (SEQ ID No.1), which encodes a protein of 1055 residues, predicted molecular weight 118,515 (PeptideSort - GCG Software) and the amino acid sequence shown in SEQ ID No.2. This sequence included the TIR domain common to Toll-like receptors and members of the interleukin-1 receptor family e.g. IL1RI, and the N-terminal sequence contains structural features as shown in figure 4. These features include, in order from the N- to the C-terminus, a predicted signal sequence with a potential cleavage site between residues 48 and 49 or 50 and 51 (SPScan in GCG; SignalP), a leucine-rich repeat motif domain, a potential transmembrane region and the Toll/IL-1R homologous region (TIR; Pfam Database). These motifs confirmed that the protein was likely to be expressed as a single transmembrane receptor-like molecule belonging to the TLR rather than the IL1R

family and therefore, it was designated TLR9 based on existing published and in-house nomenclature.

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The present invention relates to a human Toll-like receptor, referred to herein as TLR9, and variants or fragments thereof. Sequence information for TLR9 is provided in SEQ ID NO: 1 (nucleotide and amino acid) and in SEQ ID NO: 2 (amino acid only). A polypeptide of the invention consists essentially of the amino acid sequence of SEQ ID NO: 2 or of a functional variant of that sequence. One important variant of TLR9 is TLR9-A, sequence information for which is provided in SEQ ID NO: 3 (nucleotide and amino acid) and in SEQ ID NO: 4 (amino acid only).

The polypeptides are provided in isolated form. The term "isolated" is intended to convey that the polypeptide is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods. The polypeptide may be mixed with carriers or diluents which will not interfere with the intended purpose of the polypeptide and still be regarded as substantially isolated. The term "isolated" therefore includes the possibility of the polypeptide being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, expression vectors, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the polypeptide is in a state as found in nature.

A polypeptide of the invention may also be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 50%, e.g. more than 80%, 90%, 95% or 99%, by weight of the polypeptide in the preparation is a polypeptide of the invention. Routine methods, can be employed to purify and/or synthesise the proteins according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook *et al*, Molecular Cloning: a Laboratory Manual, 2nd Edition, CSH Laboratory Press (1989), the disclosure of which is included herein in its entirety by way of reference.

The term "variants" refers to polypeptides which have the same essential character or basic biological functionality as TLR9. The essential character of TLR9 can be defined as that of a Toll-like receptor. In particular, it refers to a polypeptide which has an immunomodulatory activity. In one aspect of the invention, a polypeptide of the invention may activate NFκB or may elicit pro-inflammatory cytokine production or induce expression of cell surface co-stimulatory receptors required for activation of T-cells.

Alternatively, a variant of the polypeptide of the invention is one which exhibits binding to the same ligand as TLR9. Such ligand binding may be assayed using the assays described below.

In other aspects of the invention a variant is one which does not show the same function as TLR9 but which may be used to inhibit this function. For example, a variant polypeptide for use in an assay or therapy is one which inhibits TLR9 activity, for example by inhibiting or competing out ligand binding or receptor complex formation by TLR9. Alternatively, a variant may be one which inhibits ligand binding to TLR9. Such a variant may inhibit activation of NFκB or inhibit cytokine production and expression of cell surface co-stimulatory receptors.

Such inhibitors may be used as immunomodulators to reduce inappropriate TLR activation in asthma or other chronic inflammatory diseases, or septic shock.

To determine whether a variant has the same essential function as TLR9, the immunomodulatory activity can be determined by monitoring the effect of a substance on different immune responses. For example the effect of the substance under test on NFκB activation mediated through binding the polypeptide of the present invention may be monitored. This can be carried out, for example, by co-transfection of a construct expressing the polypeptide with a construct containing a reporter gene, such as secreted placental alkaline phosphatase, under the control of a suitable NFκB-responsive promoter and monitoring for expression of the reporter gene.

Alternatively, other immunomodulatory activity such as the production of cytokines can be determined by monitoring cytokine production following incubation of a test substance with a cell expressing a polypeptide of the invention. Such assays may be carried out in the presence or absence of additional T-lymphocytes to assess the effect of such cytokines, or the direct action of a polypeptide of the invention, on such T-lymphocytes to thus determine immunomodulatory activity.

Alternatively, the Toll-like receptor functionality is as a peptide which binds a ligand of TLR9, inhibits immunomodulatory activity by TLR9 or inhibits ligand binding to TLR9 and can be determined by an assay as described below.

Preferably, a polypeptide of the invention will show the structural features associated with a Toll-like receptor. Preferably, a polypeptide of the invention, or a functional fragment thereof, contains one or more of the following structural features associated with a Toll-like receptor: an extracellular region containing leucine-rich repeat motif and cysteine-rich regions involved in ligand binding; a

single hydrophobic transmembrane region; and a C-terminal cytoplasmic domain sharing homology with other TLRs and members of the IL-1 receptor family.

Typically, polypeptides with more than about 65% identity, preferably at least 80% or at least 90% and particularly preferably at least 95%, at least 97%, or at least 99% identity, with the amino acid sequences of SEQ ID NO: 2 over a region of at least 20, preferably at least 30, at least 40, at least 60 or at least 100 contiguous amino acids or over the full length of SEQ ID NO: 2, are considered as variants of the proteins. Identity is calculated using the widely used GCG (University of Wisconsin) suite of programs and preferably using the distances software (correction method). Such variants may include allelic variants and the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the basic biological functionally of the Toll-like receptor, having a similar function to TLR9 or inhibits such function such as preventing ligand binding or TLR9 mediated activation. Such variants also include isoforms such as TLR9-A, which is 23 amino acids (or 2.2%) shorter than TLR9 (see SEQ ID NO: 2 and SEQ ID NO: 4) and thus shows 97.8 identity therewith. Transcription of the nucleotide sequence presented in SEQ ID NO:1 can result, due to variable mRNA splicing involving a second exon encoding an alternative initiating methionine, in an mRNA having the sequence of SEQ ID NO: 3 which, when translated, results in the polypeptide TLR9-A depicted in SEQ ID NO: 4.

Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 20 or 30 substitutions. The modified polypeptide generally retains activity as a TLR9 receptor or inhibitor of TLR9 receptor activity. Conservative substitutions may be made, for example according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other.

ALIPHATIC	Non-polar	G A P
		I L V
	Polar-uncharged	C S T M
		N Q
	Polar-charged	D E
		K R
AROMATIC		H F W Y

Shorter polypeptide sequences are within the scope of the invention. For example, a peptide of at least 20 amino acids or up to 50, 60, 70, 80, 100 or 150 amino acids in length is considered to fall within the scope of the invention as long as it demonstrates the basic biological functionality of TLR9 or inhibits TLR9. In accordance with this aspect of the invention the peptide may also comprise an epitope of TLR9 for generation of antibodies. In particular, but not exclusively, this aspect of the invention encompasses the situation when the protein is a fragment of the complete protein sequence and may represent a ligand-binding region (N-terminal extracellular domain) or an effector binding region (C-terminal intracellular domain). Fragments from which the C-terminus has been removed may be used as decoy receptors. Other fragments such as a secreted or soluble form of the receptor may be generated for use in an assay or in therapy in accordance with the invention. Such fragments can also be used to raise anti-TLR9 antibodies.

Polypeptides of the invention may be chemically modified, e.g. post-translationally modified. For example, they may be glycosylated or may comprise modified amino acid residues. They may also be modified by the addition of histidine residues or an epitope tag for example by a (His) 8 or (His) 6 sequence or a HA, T7, Myc or Flag tag to assist their purification or detection. They may be modified by the addition of a signal sequence to promote insertion into the

cell membrane. Such modified polypeptides fall within the scope of the term "polypeptide" of the invention.

The invention also includes nucleotide sequences that encode for TLR9 or variants thereof as well as nucleotide sequences which are complementary thereto. The nucleotide sequence may be RNA or DNA including genomic DNA, synthetic DNA or cDNA. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. Nucleotide sequence information is provided in SEQ ID NO: 1. Such nucleotides can be isolated from human cells or synthesised according to methods well known in the art, as described by way of example in Sambrook *et al.* Such nucleotides can typically be isolated from activated cells of the immune system, heart, lung, pancreatic islet cells and lymph nodes, adenoid and tonsil tissues. Figures 2 and 3 show the tissue distribution of RNA encoding TLR9, as determined by extraction of total RNA from normal or disease tissue or cells which is then used to generate cDNA for real time quantitative PCR using suitable primers and probes (TaqMan analysis) to assess expression patterns. The profiles show differential expression across tissues tested and predominance to sites containing inflammatory cell types.

Typically a polynucleotide of the invention comprises a contiguous sequence of nucleotides which is capable of hybridising under selective conditions to the coding sequence or the complement of the coding sequence of SEQ ID NO: 1.

A polynucleotide of the invention can hybridize to the coding sequence or the complement of the coding sequence of SEQ ID NO: 1 (or of SEQ ID NO: 3) at a level significantly above background. Background hybridisation may occur, for example, because of other cDNAs present in a cDNA library. The signal level generated by the interaction between a polynucleotide of the invention and the coding sequence or complement of the coding sequence of SEQ ID NO: 1 or of SEQ ID NO: 3 is typically at least 10 fold, preferably at least 100 fold, as intense

as interactions between other polynucleotides and the coding sequence of SEQ ID NO: 1 or of SEQ ID NO: 3. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P. Selective hybridisation may typically be achieved using conditions of low stringency (0.03M sodium chloride and 0.03M sodium citrate at about 40°C), medium stringency (for example, 0.03M sodium chloride and 0.03M sodium citrate at about 50°C) or high stringency (for example, 0.03M sodium chloride and 0.03M sodium citrate at about 60°C).

The coding sequence of SEQ ID NO: 1 may be modified by nucleotide substitutions, for example from 1, 2 or 3 to 10, 25, 50 or 100 substitutions. The polynucleotides of the present invention may alternatively or additionally be modified by one or more insertions and/or deletions and/or by an extension at either or both ends. The modified polynucleotide generally encodes a polypeptide which has Toll-like receptor activity or inhibits the activity of TLR9. Degenerate substitutions may be made and/or substitutions may be made which would result in a conservative amino acid substitution when the modified sequence is translated, for example as shown in the Table above.

A nucleotide sequence of the invention which is capable of selectively hybridising to the complement of the DNA coding sequence of SEQ ID NO: 1 will generally have at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity to the coding sequence of SEQ ID NO: 1 over a region of at least 20, preferably at least 30, for instance at least 40, at least 60, more preferably at least 100 contiguous nucleotides or most preferably over the full length of SEQ ID NO: 1. Methods of measuring nucleic acid and protein homology are well known in the art. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (Devereux *et al* 1984). Similarly the PILEUP and BLAST algorithms can be used to line up sequences (for example are described in Altschul 1993,

and Altschul *et al* 1990). Many different settings are possible for such programs. In accordance with the invention, the default settings may be used.

Any combination of the above mentioned degrees of sequence identity and minimum sizes may be used to define polynucleotides of the invention, with the more stringent combinations (i.e. higher sequence identity over longer lengths) being preferred. Thus, for example a polynucleotide which has at least 90% sequence identity over 25, preferably over 30 nucleotides forms one aspect of the invention, as does a polynucleotide which has at least 95% sequence identity over 40 nucleotides. The most preferred sequences have at least 70% sequence identity over at least 70% of the full length of the sequence provided by SEQ ID NO: 1.

The nucleotides according to the invention have utility in production of the proteins according to the invention, which may take place *in vitro, in vivo* or *ex vivo*. The nucleotides may be involved in recombinant protein synthesis or indeed as therapeutic agents in their own right, utilised in gene therapy techniques. Nucleotides complementary to those encoding TLR9, or antisense sequences, may also be used in gene therapy, such as in strategies for down regulation of expression of the proteins of the invention.

Polynucleotides of the invention may be used as a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors.

Such primers, probes and other fragments will preferably be at least 10, preferably at least 15 or at least 20, for example at least 25, at least 30 or at least 40 nucleotides in length. They will typically be up to 40, 50, 60, 70, 100 or 150 nucleotides in length. Probes and fragments can be longer than 150

nucleotides in length, for example up to 200, 300, 400, 500 nucleotides in length, or even up to a few nucleotides, such as five or ten nucleotides, short of the coding sequence of SEQ ID NO: 1.

The present invention also includes expression vectors that comprise nucleotide sequences encoding the proteins or variants thereof of the invention. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Other suitable vectors would be apparent to a person skilled in the art. By way of further example in this regard we refer to Sambrook *et al.*

Polynucleotides according to the invention may also be inserted into the vectors described above in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense polynucleotides may also be produced by synthetic means. Such antisense polynucleotides may be used as test compounds in the assays of the invention or may be useful in a method of treatment of the human or animal body by therapy.

Preferably, a polynucleotide of the invention or for use in the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence, such as a promoter, "operably linked" to a coding sequence is positioned in such a way that expression of the coding sequence is achieved under conditions compatible with the regulatory sequence.

The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example for the production of DNA or RNA or used to transfect or transform a host cell, for example, a mammalian host cell. The vectors may also be adapted to be used *in vivo*, for example in a method of gene therapy.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe* *nmt*1 and *adh* promoter. Mammalian promoters include the metallothionein promoter which can be induced in response to heavy metals such as cadmium. Viral promoters such as the SV40 large T antigen promoter or adenovirus promoters may also be used. All these promoters are readily available in the art.

Mammalian promoters, such as β-actin promoters, may be used. Tissue-specific promoters may be used. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR), the rous sarcoma virus (RSV) LTR promoter, the SV40 promoter, the human cytomegalovirus (CMV) IE promoter, adenovirus, HSV promoters (such as the HSV IE promoters), or HPV promoters, particularly the HPV upstream regulatory region (URR). Viral promoters are readily available in the art.

The vector may further include sequences flanking the polynucleotide which comprise sequences homologous to eukaryotic genomic sequences, preferably mammalian genomic sequences, or viral genomic sequences. This will allow the

introduction of the polynucleotides of the invention into the genome of eukaryotic cells or viruses by homologous recombination. In particular, a plasmid vector comprising the expression cassette flanked by viral sequences can be used to prepare a viral vector suitable for delivering the polynucleotides of the invention to a mammalian cell. Other examples of suitable viral vectors include herpes simplex viral vectors and retroviruses, including lentiviruses, adenoviruses, adeno-associated viruses and HPV viruses (such as HPV-16 or HPV-18). Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide giving rise to the RNA into the host genome. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression.

The invention also includes cells that have been modified to express the TLR9 polypeptide or a variant thereof. Such cells include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which may be modified by insertion of vectors encoding for a polypeptide according to the invention include mammalian HEK293T, CHO, HeLa and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of a polypeptide. Cells such as T-cells, monocytes or dendritic cells expressing the receptor may be used for example in screening. Expression may be achieved in transformed oocytes. A polypeptide of the invention may be expressed in cells of a transgenic non-human animal, preferably a mouse. A transgenic non-human animal expressing a polypeptide of the invention is included within the scope of the invention.

It is also possible for the proteins of the invention to be transiently expressed in a cell line or on a membrane, such as for example in a baculovirus expression

system. Such systems, which are adapted to express the proteins according to the invention, are also included within the scope of the present invention.

According to another aspect, the present invention also relates to antibodies (either polyclonal or preferably monoclonal antibodies, chimeric, single chain, Fab fragments) which have been raised by standard techniques and are specific for a polypeptide of the invention. Such antibodies could for example, be useful in purification, isolation or screening methods involving immunoprecipitation techniques and may be used as tools to further elucidate the function of TLR9 or a variant thereof, or indeed as therapeutic agents in their own right. Antibodies may also be raised against specific epitopes of the proteins according to the invention. Such antibodies may be used to block ligand binding to the receptor. Alternatively an antibody may be provided which acts as an agonist, to cross link receptors of the invention to mediate receptor activity. An antibody, or other compound, "specifically binds" to a protein when it binds with high affinity to the protein for which it is specific but does not bind or binds with only low affinity to other proteins. A variety of protocols for competitive binding or immunoradiometric assays to determine the specific binding capability of an antibody are well known in the art (see for example Maddox *et al* 1993). Such immunoassays typically involve the formation of complexes between the "specific protein" and its antibody and the measurement of complex formation.

An important aspect of the present invention is the use of polypeptides according to the invention in screening methods to identify compounds that may act as modulators of Toll-like receptor activity. Any suitable form may be used for the assay to identify a modulator of TLR9 activity. In general terms, such screening methods may involve contacting a polypeptide of the invention with a test compound and then measuring receptor activity.

Screening methods may alternatively involve contacting a polypeptide of the invention with a test compound and then monitoring for the effect on immunomodulatory activity.

The binding of the substance to a polypeptide in the invention can be determined directly. For example, a radiolabelled test substance can be incubated with a polypeptide of the invention and so that binding of the test substance to the polypeptide can be monitored. Typically, the radiolabelled test substance can be incubated with cell membranes containing the polypeptide until equilibrium is reached. The membranes can then be separated from a non-bound test substance and dissolved in scintillation fluid to allow the radioactive content to be determined by scintillation counting. Non-specific binding of the test substance may also be determined by repeating the experiments in the presence of a saturating concentration of a non-radioactive ligand. Preferably, a binding curve is constructed by repeating the experiment with various concentrations of the test substance.

Cell based assays may also be carried out, for example using a cell expressing the TLR9 receptor, and contacting the cell with another cell to look for ligand binding or activation of TLR9-mediated pathways such as NFKB activation. Alternatively cells expressing TLR9 constitutively may be provided for use in assays for TLR9 function. Such constitutively expressed TLR9 may demonstrate TLR9 activity in the absence of ligand binding. Additional test substances may be introduced in any assay to look for inhibitors of ligand binding or inhibitors of TLR9-mediated activity. Assays are preferably carried out using cells expressing TLR9, and incubating such cells with the test substance optionally in the presence of TLR9 ligand. Alternatively an antibody may be used to complex TLR9 and thus mediate TLR9-activity. Test substances may then be added to assess the effect on such activity.

In preferred aspects, a host cell is provided expressing the receptor, or a receptor complex of TLR9 (or TLR9-A) comprising a homodimer, a heterodimer of TLR9 (or TLR9-A) with another Toll-like receptor, or a complex of TLR9 (or TLR9-A) with protein cofactors, and containing an NFκB responsive reporter construct. The host cell is treated with a substance under test for a defined time. The expression of the reporter gene, such as secreted placental (SP) alkaline phosphatase or luciferase is assayed. The assay enables determination of whether the addition of compounds inhibits the induction of the response in target cells. Alternatively the assay may be carried out to identify cytokine production or it may be carried out in the presence of T-cells to identify inducement of co-stimulatory receptors required for activation of T-cells.

Assays may also be carried out to identify modulators of receptor shedding. A polypeptide of the invention can be cleaved from the cell surface. Shedding the receptor would act to down regulate receptor signalling. Thus, cell based assays may be used to screen for compounds which promote or inhibit receptor-shedding. Assays may also be carried out to identify substances which modify TLR9 receptor expression for example substances which down regulate expression. Such assays may be carried out for example by using antibodies for TLR9 to monitor levels of TLR9 expression.

Additional control experiments may be carried out. Assays may also be carried out using known ligands of other Toll-like receptors to identify ligands which are specific for polypeptides of the invention. Preferably, the assays of the invention are carried out under conditions which would result in immunomodulatory NFκB mediated activity in the absence of the test substance, to identify inhibitors of Toll-like receptor mediated activity, or agents which inhibit ligand-induced Toll-like receptor activity.

Suitable test substances which can be tested in the above assays include combinatorial libraries, defined chemical entities, peptides and peptide mimetics, oligonucleotides and natural product libraries, such as display (e.g. phage display libraries) and antibody products. In a preferred embodiment, the test substance is a variant peptide of the invention. In particularly preferred embodiments, suitable test substances which may be candidate ligands for binding to and modulation of TLR9 or TLR9-A include synthetic or naturally occurring oligonucleotides or polynucleotides which bind directly to the Toll-like receptor or which modulate the Toll-like receptor of the present invention via one or more intermediate signal transducers. Such oligo- or polynucleotides may be "CG-rich" sequences or sequences including one or more unmethylated CpG nucleotide pairs.

The assay may be carried out using full length TLR9 to identify a variant peptide which interferes with TLR9 mediated activity, for example by inhibiting ligand binding.

Test substances may be used in an initial screen of, for example, 10 substances per reaction, and the substances of these batches which show inhibition or activation tested individually. Test substances may be used at a concentration of from 1nM to 1000μM, preferably from 1μM to 100μM, more preferably from 1μM to 10μM.

A protein-binding assay may be developed using a polypeptide of the invention, preferably one encoding the extracellular ligand-binding domain, to identify novel protein ligands of TLR9. Particular examples may be screening of a human cDNA expression library for protein ligands of TLR9 by yeast 2-hybrid protein interaction assay (e.g. as described in International Patent Application No. WO99/49294).

Another aspect of the present invention is the use of polynucleotides encoding the TLR9 polypeptides of the invention to identify mutations in TLR9 genes which may be implicated in human disorders or to identify cells in which TLR9 is expressed. Identification of such mutations may be used to assist in diagnosis of immune system, lung, kidney, heart or other disorders or susceptibility to such disorders and in assessing the physiology of such disorders. In particular the polynucleotides of the invention may assist in diagnosis of asthma and rheumatoid arthritis. For example, a SNP (single nucleotide polymorphism) has been identified in the genomic DNA encoding TLR9 (G/A nucleotide: The SNP Consortium database accession number TSC0164834). This single base pair change lies in the DNA region encoding the 23 N-terminal residues of TLR9, and this region is spliced out of the mRNA encoding TLR9-A. The nucleotide at this SNP position may affect the efficiency of mRNA splicing in the two different variants - a G at this position may possibly disrupt the splicing machinery and an A might lead to more efficient splicing. Additionally, the presence of a G as compared to an A in an unspliced mRNA would introduce a stop codon and result in different N terminal protein sequences upon translation of that mRNA, thus the two polymorphic variants of the tlr9 gene may encode receptors which have differing expression levels and/or differing functional activity levels. The present invention provides a diagnostic tool for determining the polymorphic variant in an individual by detecting the DNA sequence at the SNP site. Such a tool may incorporate a nucleotide probe specific for one or other of the polymorphic variants, for example an oligonucleotide of from 5 to 50, preferably 5-20 nucleotides, complementary to a fragment of the nucleotide sequence of SEQ ID No. 1 which extends over the SNP site or a fragment complementary to that sequence with the exception of the single nucleotide change (G to A) at the SNP site. The present invention also provides a method of detecting a polymorphic variant in the tlr9 gene by determining the sequence of nucleotides at and around the SNP site identified by the SNP consortium database accession number

TSC0164834, in particular by determining whether the nucleotide at that SNP site is a G or an A.

Another aspect of the present invention is the use of the compounds that have been identified by screening techniques referred to above in the treatment or prophylaxis of disorders which are responsive to regulation of TLR9 receptor activity. In addition, variant peptides of the invention which inhibit TLR9-mediated activity, for example which inhibit ligand binding or prevent hTLR9 immunomodulatory activity may be used in the treatment or prophylaxis of such disorders. Antibodies which recognise TLR9 may similarly be used in therapy.

In particular, such compounds may be used in the treatment of inflammatory, cardiovascular, systemic infection or autoimmune disease. The compounds may be used to treat bacterial, viral or fungal infections, asthma, rhinitis, chronic obstructive pulmonary disease (COPD), emphysema, an inflammatory bowel disease such as ulcerative colitis and Crohn's disease, rheumatoid arthritis, osteoarthritis, psoriasis, Alzheimers disease, atherosclerosis, septic shock syndrome associated with systemic infection involving gram positive and gram negative bacteria, diabetes, Multiple Sclerosis.

In an alternative aspect, the invention provides agents which activate TLR9 mediated immunomodulation for use as an immunoadjuvant, or TLR9, and variants thereof, or polynucleotides or DNA encoding a polypeptide of the invention which may be administered for use as immunoadjuvants to enhance or alter the immune response in an individual to an antigen.

The compounds identified according to the screening methods outlined above may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art, and as fully described in Remmington's Pharmaceutical Sciences, Mack Publishing Company, Eastern

Pennsylvania 17th Ed. 1985, the disclosure of which is included herein of its entirety by way of reference.

The compounds may be administered by enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intra-arterial, intramuscular, intraperitoneal, topical or other appropriate administration routes. The dose of a compound to be administered may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg of body weight, according to the activity of the compound, the age, weight and conditions of the subject to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g.

Nucleic acid encoding an inhibitor of TLR9 activity may be administered to the mammal. In an alternative aspect of the invention, nucleic acid encoding TLR9 or a variant thereof may be administered to the animal. Such a variant shows immunomodulatory activity of TLR9 such as inducing cytokine production and expression of cell surface co-stimulatory receptors required for activation of T-cells. Nucleic acid, such as RNA or DNA, and preferably, DNA, is provided in the form of a vector, such as the polynucleotides described above, which may be expressed in the cells of the mammal.

Nucleic acid encoding the peptide may be administered to the animal by any available technique. For example, the nucleic acid may be introduced by injection, preferably intradermally, subcutaneously or intramuscularly. Alternatively, the nucleic acid may be delivered directly across the skin using a nucleic acid delivery device such as particle-mediated gene delivery. The

cells. Tertiary screens involve the study of modulators in rat and mouse models of disease relevant to the target.

5

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Gln Thr Leu Arg Leu Gln Met Asn Phe Ile Asn Gln Ala Gln Leu Gly
            420                 425                 430
Ile Phe Arg Ala Phe Pro Gly Leu Arg Tyr Val Asp Leu Ser Asp Asn
        435                 440                 445
Arg Ile Ser Gly Ala Ser Glu Leu Thr Ala Thr Met Gly Glu Ala Asp
    450                 455                 460
Gly Gly Glu Lys Val Trp Leu Gln Pro Gly Asp Leu Ala Pro Ala Pro
465                 470                 475                 480
Val Asp Thr Pro Ser Ser Glu Asp Phe Arg Pro Asn Cys Ser Thr Leu
                485                 490                 495
Asn Phe Thr Leu Asp Leu Ser Arg Asn Asn Leu Val Thr Val Gln Pro
            500                 505                 510
Glu Met Phe Ala Gln Leu Ser His Leu Gln Cys Leu Arg Leu Ser His
        515                 520                 525
Asn Cys Ile Ser Gln Ala Val Asn Gly Ser Gln Phe Leu Pro Leu Thr
    530                 535                 540
Gly Leu Gln Val Leu Asp Leu Ser His Asn Lys Leu Asp Leu Tyr His
545                 550                 555                 560
Glu His Ser Phe Thr Glu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ser
                565                 570                 575
Tyr Asn Ser Gln Pro Phe Gly Met Gln Gly Val Gly His Asn Phe Ser
            580                 585                 590
Phe Val Ala His Leu Arg Thr Leu Arg His Leu Ser Leu Ala His Asn
        595                 600                 605
Asn Ile His Ser Gln Val Ser Gln Gln Leu Cys Ser Thr Ser Leu Arg
    610                 615                 620
Ala Leu Asp Phe Ser Gly Asn Ala Leu Gly His Met Trp Ala Glu Gly
625                 630                 635                 640
Asp Leu Tyr Leu His Phe Phe Gln Gly Leu Ser Gly Leu Ile Trp Leu
                645                 650                 655
```

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Asp Leu Ser Gln Asn Arg Leu His Thr Leu Leu Pro Gln Thr Leu Arg
            660                 665                 670
Asn Leu Pro Lys Ser Leu Gln Val Leu Arg Leu Arg Asp Asn Tyr Leu
        675                 680                 685
Ala Phe Phe Lys Trp Trp Ser Leu His Phe Leu Pro Lys Leu Glu Val
    690                 695                 700
Leu Asp Leu Ala Gly Asn Gln Leu Lys Ala Leu Thr Asn Gly Ser Leu
705                 710                 715                 720
Pro Ala Gly Thr Arg Leu Arg Arg Leu Asp Val Ser Cys Asn Ser Ile
                725                 730                 735
Ser Phe Val Ala Pro Gly Phe Phe Ser Lys Ala Lys Glu Leu Arg Glu
            740                 745                 750
Leu Asn Leu Ser Ala Asn Ala Leu Lys Thr Val Asp His Ser Trp Phe
        755                 760                 765
Gly Pro Leu Ala Ser Ala Leu Gln Ile Leu Asp Val Ser Ala Asn Pro
    770                 775                 780
Leu His Cys Ala Cys Gly Ala Ala Phe Met Asp Phe Leu Leu Glu Val
785                 790                 795                 800
Gln Ala Ala Val Pro Gly Leu Pro Ser Arg Val Lys Cys Gly Ser Pro
                805                 810                 815
Gly Gln Leu Gln Gly Leu Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys
            820                 825                 830
Leu Asp Glu Ala Leu Ser Trp Asp Cys Phe Ala Leu Ser Leu Leu Ala
        835                 840                 845
Val Ala Leu Gly Leu Gly Val Pro Met Leu His His Leu Cys Gly Trp
    850                 855                 860
Asp Leu Trp Tyr Cys Phe His Leu Cys Leu Ala Trp Leu Pro Trp Arg
865                 870                 875                 880
Gly Arg Gln Ser Gly Arg Asp Glu Asp Ala Leu Pro Tyr Asp Ala Phe
                885                 890                 895
Val Val Phe Asp Lys Thr Gln Ser Ala Val Ala Asp Trp Val Tyr Asn
            900                 905                 910
Glu Leu Arg Gly Gln Leu Glu Glu Cys Arg Gly Arg Trp Ala Leu Arg
        915                 920                 925
Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro Gly Lys Thr Leu Phe Glu
    930                 935                 940
Asn Leu Trp Ala Ser Val Tyr Gly Ser Arg Lys Thr Leu Phe Val Leu
945                 950                 955                 960
Ala His Thr Asp Arg Val Ser Gly Leu Leu Arg Ala Ser Phe Leu Leu
                965                 970                 975
Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys Asp Val Val Val Leu Val
            980                 985                 990
Ile Leu Ser Pro Asp Gly Arg Arg Ser Arg Tyr Val Arg Leu Arg Gln
        995                1000                1005
Arg Leu Cys Arg Gln Ser Val Leu Leu Trp Pro His Gln Pro Ser Gly
   1010                1015                1020
Gln Arg Ser Phe Trp Ala Gln Leu Gly Met Ala Leu Thr Arg Asp Asn
1025                1030                1035                1040
His His Phe Tyr Asn Arg Asn Phe Cys Gln Gly Pro Thr Ala Glu
                1045                1050                1055
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Seq.id.no.3

```
atg ggt ttc tgc cgc agc gcc ctg cac
met Gly Phe Cys Arg Ser Ala Leu His
                5
ccg ctg tct ctc ctg gtg cag gcc atc atg ctg gcc atg acc ctg gcc   75
Pro Leu Ser Leu Leu Val Gln Ala Ile Met Leu Ala Met Thr Leu Ala
 10                  15                  20                  25
ctg ggt acc ttg cct gcc ttc cta ccc tgt gag ctc cag ccc cac ggc   123
Leu Gly Thr Leu Pro Ala Phe Leu Pro Cys Glu Leu Gln Pro His Gly
            30                  35                  40
ctg gtg aac tgc aac tgg ctg ttc ctg aag tct gtg ccc cac ttc tcc   171
Leu Val Asn Cys Asn Trp Leu Phe Leu Lys Ser Val Pro His Phe Ser
        45                  50                  55
atg gca gca ccc cgt ggc aat gtc acc agc ctt tcc ttg tcc tcc aac   219
Met Ala Ala Pro Arg Gly Asn Val Thr Ser Leu Ser Leu Ser Ser Asn
    60                  65                  70
cgc atc cac cac ctc cat gat tct gac ttt gcc cac ctg ccc agc ctg   267
Arg Ile His His Leu His Asp Ser Asp Phe Ala His Leu Pro Ser Leu
 75                  80                  85
cgg cat ctc aac ctc aag tgg aac tgc ccg ccg gtt ggc ctc agc ccc   315
Arg His Leu Asn Leu Lys Trp Asn Cys Pro Pro Val Gly Leu Ser Pro
 90                  95                  100                 105
atg cac ttc ccc tgc cac atg acc atc gag ccc agc acc ttc ttg gct   363
Met His Phe Pro Cys His Met Thr Ile Glu Pro Ser Thr Phe Leu Ala
            110                 115                 120
gtg ccc acc ctg gaa gag cta aac ctg agc tac aac aac atc atg act   411
Val Pro Thr Leu Glu Glu Leu Asn Leu Ser Tyr Asn Asn Ile Met Thr
        125                 130                 135
gtg cct gcg ctg ccc aaa tcc ctc ata tcc ctg tcc ctc agc cat acc   459
Val Pro Ala Leu Pro Lys Ser Leu Ile Ser Leu Ser Leu Ser His Thr
    140                 145                 150
aac atc ctg atg cta gac tct gcc agc ctc gcc ggc ctg cat gcc ctg   507
Asn Ile Leu Met Leu Asp Ser Ala Ser Leu Ala Gly Leu His Ala Leu
155                 160                 165
cgc ttc cta ttc atg gac ggc aac tgt tat tac aag aac ccc tgc agg   555
Arg Phe Leu Phe Met Asp Gly Asn Cys Tyr Tyr Lys Asn Pro Cys Arg
170                 175                 180                 185
cag gca ctg gag gtg gcc ccg ggt gcc ctc ctt ggc ctg ggc aac ctc   603
Gln Ala Leu Glu Val Ala Pro Gly Ala Leu Leu Gly Leu Gly Asn Leu
            190                 195                 200
acc cac ctg tca ctc aag tac aac aac ctc act gtg gtg ccc cgc aac   651
Thr His Leu Ser Leu Lys Tyr Asn Asn Leu Thr Val Val Pro Arg Asn
        205                 210                 215
ctg cct tcc agc ctg gag tat ctg ctg ttg tcc tac aac cgc atc gtc   699
Leu Pro Ser Ser Leu Glu Tyr Leu Leu Leu Ser Tyr Asn Arg Ile Val
    220                 225                 230
aaa ctg gcg cct gag gac ctg gcc aat ctg acc gcc ctg cgt gtg ctc   747
Lys Leu Ala Pro Glu Asp Leu Ala Asn Leu Thr Ala Leu Arg Val Leu
235                 240                 245
gat gtg ggc gga aat tgc cgc cgc tgc gac cac gct ccc aac ccc tgc   795
Asp Val Gly Gly Asn Cys Arg Arg Cys Asp His Ala Pro Asn Pro Cys
250                 255                 260                 265
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atg gag tgc cct cgt cac ttc ccc cag cta cat ccc gat acc ttc agc   843
Met Glu Cys Pro Arg His Phe Pro Gln Leu His Pro Asp Thr Phe Ser
                270                 275                 280
cac ctg agc cgt ctt gaa ggc ctg gtg ttg aag gac agt tct ctc tcc   891
His Leu Ser Arg Leu Glu Gly Leu Val Leu Lys Asp Ser Ser Leu Ser
            285                 290                 295
tgg ctg aat gcc agt tgg ttc cgt ggg ctg gga aac ctc cga gtg ctg   939
Trp Leu Asn Ala Ser Trp Phe Arg Gly Leu Gly Asn Leu Arg Val Leu
        300                 305                 310
gac ctg agt gag aac ttc ctc tac aaa tgc atc act aaa acc aag gcc   987
Asp Leu Ser Glu Asn Phe Leu Tyr Lys Cys Ile Thr Lys Thr Lys Ala
    315                 320                 325
ttc cag ggc cta aca cag ctg cgc aag ctt aac ctg tcc ttc aat tac  1035
Phe Gln Gly Leu Thr Gln Leu Arg Lys Leu Asn Leu Ser Phe Asn Tyr
330                 335                 340                 345
caa aag agg gtg tcc ttt gcc cac ctg tct ctg gcc cct tcc ttc ggg  1083
Gln Lys Arg Val Ser Phe Ala His Leu Ser Leu Ala Pro Ser Phe Gly
                350                 355                 360
agc ctg gtc gcc ctg aag gag ctg gac atg cac ggc atc ttc ttc cgc  1131
Ser Leu Val Ala Leu Lys Glu Leu Asp Met His Gly Ile Phe Phe Arg
            365                 370                 375
tca ctc gat gag acc acg ctc cgg cca ctg gcc cgc ctg ccc atg ctc  1179
Ser Leu Asp Glu Thr Thr Leu Arg Pro Leu Ala Arg Leu Pro Met Leu
        380                 385                 390
cag act ctg cgt ctg cag atg aac ttc atc aac cag gcc cag ctc ggc  1127
Gln Thr Leu Arg Leu Gln Met Asn Phe Ile Asn Gln Ala Gln Leu Gly
    395                 400                 405
atc ttc agg gcc ttc cct ggc ctg cgc tac gtg gac ctg tcg gac aac  1275
Ile Phe Arg Ala Phe Pro Gly Leu Arg Tyr Val Asp Leu Ser Asp Asn
410                 415                 420                 425
cgc atc agc gga gct tcg gag ctg aca gcc acc atg ggg gag gca gat  1323
Arg Ile Ser Gly Ala Ser Glu Leu Thr Ala Thr Met Gly Glu Ala Asp
                430                 435                 440
gga ggg gag aag gtc tgg ctg cag cct ggg gac ctt gct ccg gcc cca  1371
Gly Gly Glu Lys Val Trp Leu Gln Pro Gly Asp Leu Ala Pro Ala Pro
            445                 450                 455
gtg gac act ccc agc tct gaa gac ttc agg ccc aac tgc agc acc ctc  1419
Val Asp Thr Pro Ser Ser Glu Asp Phe Arg Pro Asn Cys Ser Thr Leu
        460                 465                 470
aac ttc acc ttg gat ctg tca cgg aac aac ctg gtg acc gtg cag ccg  1467
Asn Phe Thr Leu Asp Leu Ser Arg Asn Asn Leu Val Thr Val Gln Pro
    475                 480                 485
gag atg ttt gcc cag ctc tcg cac ctg cag tgc ctg cgc ctg agc cac  1515
Glu Met Phe Ala Gln Leu Ser His Leu Gln Cys Leu Arg Leu Ser His
490                 495                 500                 505
aac tgc atc tcg cag gca gtc aat ggc tcc cag ttc ctg ccg ctg acc  1563
Asn Cys Ile Ser Gln Ala Val Asn Gly Ser Gln Phe Leu Pro Leu Thr
                510                 515                 520
ggt ctg cag gtg cta gac ctg tcc cac aat aag ctg gac ctc tac cac  1611
Gly Leu Gln Val Leu Asp Leu Ser His Asn Lys Leu Asp Leu Tyr His
            525                 530                 535
gag cac tca ttc acg gag cta cca cga ctg gag gcc ctg gac ctc agc  1659
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Glu His Ser Phe Thr Glu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ser
        540                 545                 550
tac aac agc cag ccc ttt ggc atg cag ggc gtg ggc cac aac ttc agc   1707
Tyr Asn Ser Gln Pro Phe Gly Met Gln Gly Val Gly His Asn Phe Ser
    555                 560                 565
ttc gtg gct cac ctg cgc acc ctg cgc cac ctc agc ctg gcc cac aac   1755
Phe Val Ala His Leu Arg Thr Leu Arg His Leu Ser Leu Ala His Asn
570                 575                 580                 585
aac atc cac agc caa gtg tcc cag cag ctc tgc agt acg tcg ctg cgg   1803
Asn Ile His Ser Gln Val Ser Gln Gln Leu Cys Ser Thr Ser Leu Arg
                590                 595                 600
gcc ctg gac ttc agc ggc aat gca ctg ggc cat atg tgg gcc gag gga   1851
Ala Leu Asp Phe Ser Gly Asn Ala Leu Gly His Met Trp Ala Glu Gly
            605                 610                 615
gac ctc tat ctg cac ttc ttc caa ggc ctg agc ggt ttg atc tgg ctg   1899
Asp Leu Tyr Leu His Phe Phe Gln Gly Leu Ser Gly Leu Ile Trp Leu
        620                 625                 630
gac ttg tcc cag aac cgc ctg cac acc ctc ctg ccc caa acc ctg cgc   1947
Asp Leu Ser Gln Asn Arg Leu His Thr Leu Leu Pro Gln Thr Leu Arg
    635                 640                 645
aac ctc ccc aag agc cta cag gtg ctg cgt ctc cgt gac aat tac ctg   1995
Asn Leu Pro Lys Ser Leu Gln Val Leu Arg Leu Arg Asp Asn Tyr Leu
650                 655                 660                 665
gcc ttc ttt aag tgg tgg agc ctc cac ttc ctg ccc aaa ctg gaa gtc   2043
Ala Phe Phe Lys Trp Trp Ser Leu His Phe Leu Pro Lys Leu Glu Val
                670                 675                 680
ctc gac ctg gca gga aac cag ctg aag gcc ctg acc aat ggc agc ctg   2091
Leu Asp Leu Ala Gly Asn Gln Leu Lys Ala Leu Thr Asn Gly Ser Leu
            685                 690                 695
cct gct ggc acc cgg ctc cgg agg ctg gat gtc agc tgc aac agc atc   2139
Pro Ala Gly Thr Arg Leu Arg Arg Leu Asp Val Ser Cys Asn Ser Ile
        700                 705                 710
agc ttc gtg gcc ccc ggc ttc ttt tcc aag gcc aag gag ctg cga gag   2187
Ser Phe Val Ala Pro Gly Phe Phe Ser Lys Ala Lys Glu Leu Arg Glu
    715                 720                 725
ctc aac ctt agc gcc aac gcc ctc aag aca gtg gac cac tcc tgg ttt   2235
Leu Asn Leu Ser Ala Asn Ala Leu Lys Thr Val Asp His Ser Trp Phe
730                 735                 740                 745
ggg ccc ctg gcg agt gcc ctg caa ata cta gat gta agc gcc aac cct   2283
Gly Pro Leu Ala Ser Ala Leu Gln Ile Leu Asp Val Ser Ala Asn Pro
                750                 755                 760
ctg cac tgc gcc tgt ggg gcg gcc ttt atg gac ttc ctg ctg gag gtg   2331
Leu His Cys Ala Cys Gly Ala Ala Phe Met Asp Phe Leu Leu Glu Val
            765                 770                 775
cag gct gcc gtg ccc ggt ctg ccc agc cgg gtg aag tgt ggc agt ccg   2379
Gln Ala Ala Val Pro Gly Leu Pro Ser Arg Val Lys Cys Gly Ser Pro
        780                 785                 790
ggc cag ctc cag ggc ctc agc atc ttt gca cag gac ctg cgc ctc tgc   2427
Gly Gln Leu Gln Gly Leu Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys
    795                 800                 805
ctg gat gag gcc ctc tcc tgg gac tgt ttc gcc ctc tcg ctg ctg gct   2475
Leu Asp Glu Ala Leu Ser Trp Asp Cys Phe Ala Leu Ser Leu Leu Ala
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810                 815                 820                 825
gtg gct ctg ggc ctg ggt gtg ccc atg ctg cat cac ctc tgt ggc tgg   2523
Val Ala Leu Gly Leu Gly Val Pro Met Leu His His Leu Cys Gly Trp
                830                 835                 840
gac ctc tgg tac tgc ttc cac ctg tgc ctg gcc tgg ctt ccc tgg cgg   2571
Asp Leu Trp Tyr Cys Phe His Leu Cys Leu Ala Trp Leu Pro Trp Arg
            845                 850                 855
ggg cgg caa agt ggg cga gat gag gat gcc ctg ccc tac gat gcc ttc   2619
Gly Arg Gln Ser Gly Arg Asp Glu Asp Ala Leu Pro Tyr Asp Ala Phe
        860                 865                 870
gtg gtc ttc gac aaa acg cag agc gca gtg gca gac tgg gtg tac aac   2667
Val Val Phe Asp Lys Thr Gln Ser Ala Val Ala Asp Trp Val Tyr Asn
    875                 880                 885
gag ctt cgg ggg cag ctg gag gag tgc cgt ggg cgc tgg gca ctc cgc   2715
Glu Leu Arg Gly Gln Leu Glu Glu Cys Arg Gly Arg Trp Ala Leu Arg
890                 895                 900                 905
ctg tgc ctg gag gaa cgc gac tgg ctg cct ggc aaa acc ctc ttt gag   2763
Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro Gly Lys Thr Leu Phe Glu
                910                 915                 920
aac ctg tgg gcc tcg gtc tat ggc agc cgc aag acg ctg ttt gtg ctg   2811
Asn Leu Trp Ala Ser Val Tyr Gly Ser Arg Lys Thr Leu Phe Val Leu
            925                 930                 935
gcc cac acg gac cgg gtc agt ggt ctc ttg cgc gcc agc ttc ctg ctg   2859
Ala His Thr Asp Arg Val Ser Gly Leu Leu Arg Ala Ser Phe Leu Leu
        940                 945                 950
gcc cag cag cgc ctg ctg gag gac cgc aag gac gtc gtg gtg ctg gtg   2907
Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys Asp Val Val Val Leu Val
    955                 960                 965
atc ctg agc cct gac ggc cgc cgc tcc cgc tat gtg cgg ctg cgc cag   2955
Ile Leu Ser Pro Asp Gly Arg Arg Ser Arg Tyr Val Arg Leu Arg Gln
970                 975                 980                 985
cgc ctc tgc cgc cag agt gtc ctc ctg tgg ccc cac cag ccc agt ggt   3003
Arg Leu Cys Arg Gln Ser Val Leu Leu Trp Pro His Gln Pro Ser Gly
                990                 995                1000
cag cgc agc ttc tgg gcc cag ctg ggc atg gcc ctg acc agg gac aac   3051
Gln Arg Ser Phe Trp Ala Gln Leu Gly Met Ala Leu Thr Arg Asp Asn
            1005                1010                1015
cac cac ttc tat aac cgg aac ttc tgc cag gga ccc acg gcc gaa       3096
His His Phe Tyr Asn Arg Asn Phe Cys Gln Gly Pro Thr Ala Glu
        1020                1025                1030
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Seq.Id.No.4

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Met Gly Phe Cys Arg Ser Ala Leu His
                  5
Pro Leu Ser Leu Leu Val Gln Ala Ile Met Leu Ala Met Thr Leu Ala
10                 15                  20                  25
Leu Gly Thr Leu Pro Ala Phe Leu Pro Cys Glu Leu Gln Pro His Gly
                30                  35                  40
Leu Val Asn Cys Asn Trp Leu Phe Leu Lys Ser Val Pro His Phe Ser
```

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                45                  50                  55
Met Ala Ala Pro Arg Gly Asn Val Thr Ser Leu Ser Leu Ser Ser Asn
            60                  65                  70
Arg Ile His His Leu His Asp Ser Asp Phe Ala His Leu Pro Ser Leu
        75                  80                  85
Arg His Leu Asn Leu Lys Trp Asn Cys Pro Pro Val Gly Leu Ser Pro
    90                  95                  100                 105
Met His Phe Pro Cys His Met Thr Ile Glu Pro Ser Thr Phe Leu Ala
                110                 115                 120
Val Pro Thr Leu Glu Glu Leu Asn Leu Ser Tyr Asn Asn Ile Met Thr
            125                 130                 135
Val Pro Ala Leu Pro Lys Ser Leu Ile Ser Leu Ser Leu Ser His Thr
        140                 145                 150
Asn Ile Leu Met Leu Asp Ser Ala Ser Leu Ala Gly Leu His Ala Leu
    155                 160                 165
Arg Phe Leu Phe Met Asp Gly Asn Cys Tyr Tyr Lys Asn Pro Cys Arg
170                 175                 180                 185
Gln Ala Leu Glu Val Ala Pro Gly Ala Leu Leu Gly Leu Gly Asn Leu
                190                 195                 200
Thr His Leu Ser Leu Lys Tyr Asn Asn Leu Thr Val Val Pro Arg Asn
            202                 210                 215
Leu Pro Ser Ser Leu Glu Tyr Leu Leu Leu Ser Tyr Asn Arg Ile Val
        220                 225                 230
Lys Leu Ala Pro Glu Asp Leu Ala Asn Leu Thr Ala Leu Arg Val Leu
    235                 240                 245
Asp Val Gly Gly Asn Cys Arg Arg Cys Asp His Ala Pro Asn Pro Cys
250                 255                 260                 265
Met Glu Cys Pro Arg His Phe Pro Gln Leu His Pro Asp Thr Phe Ser
                270                 275                 280
His Leu Ser Arg Leu Glu Gly Leu Val Leu Lys Asp Ser Ser Leu Ser
            285                 290                 295
Trp Leu Asn Ala Ser Trp Phe Arg Gly Leu Gly Asn Leu Arg Val Leu
        300                 305                 310
Asp Leu Ser Glu Asn Phe Leu Tyr Lys Cys Ile Thr Lys Thr Lys Ala
    315                 320                 325
Phe Gln Gly Leu Thr Gln Leu Arg Lys Leu Asn Leu Ser Phe Asn Tyr
330                 335                 340                 345
Gln Lys Arg Val Ser Phe Ala His Leu Ser Leu Ala Pro Ser Phe Gly
                350                 355                 360
Ser Leu Val Ala Leu Lys Glu Leu Asp Met His Gly Ile Phe Phe Arg
            365                 370                 375
Ser Leu Asp Glu Thr Thr Leu Arg Pro Leu Ala Arg Leu Pro Met Leu
        380                 385                 390
Gln Thr Leu Arg Leu Gln Met Asn Phe Ile Asn Gln Ala Gln Leu Gly
    395                 400                 405
Ile Phe Arg Ala Phe Pro Gly Leu Arg Tyr Val Asp Leu Ser Asp Asn
410                 415                 420                 425
Arg Ile Ser Gly Ala Ser Glu Leu Thr Ala Thr Met Gly Glu Ala Asp
                430                 435                 440
Gly Gly Glu Lys Val Trp Leu Gln Pro Gly Asp Leu Ala Pro Ala Pro
            445                 450                 455
Val Asp Thr Pro Ser Ser Glu Asp Phe Arg Pro Asn Cys Ser Thr Leu
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460 465 470
Asn Phe Thr Leu Asp Leu Ser Arg Asn Asn Leu Val Thr Val Gln Pro
475 480 485
Glu Met Phe Ala Gln Leu Ser His Leu Gln Cys Leu Arg Leu Ser His
490 495 500 505
Asn Cys Ile Ser Gln Ala Val Asn Gly Ser Gln Phe Leu Pro Leu Thr
510 515 520
Gly Leu Gln Val Leu Asp Leu Ser His Asn Lys Leu Asp Leu Tyr His
525 530 535
Glu His Ser Phe Thr Glu Leu Pro Arg Leu Glu Ala Leu Asp Leu Ser
540 545 550
Tyr Asn Ser Gln Pro Phe Gly Met Gln Gly Val Gly His Asn Phe Ser
555 560 565
Phe Val Ala His Leu Arg Thr Leu Arg His Leu Ser Leu Ala His Asn
570 575 580 585
Asn Ile His Ser Gln Val Ser Gln Gln Leu Cys Ser Thr Ser Leu Arg
590 595 600
Ala Leu Asp Phe Ser Gly Asn Ala Leu Gly His Met Trp Ala Glu Gly
605 610 615
Asp Leu Tyr Leu His Phe Phe Gln Gly Leu Ser Gly Leu Ile Trp Leu
620 625 630
Asp Leu Ser Gln Asn Arg Leu His Thr Leu Leu Pro Gln Thr Leu Arg
635 640 645
Asn Leu Pro Lys Ser Leu Gln Val Leu Arg Leu Arg Asp Asn Tyr Leu
650 655 660 665
Ala Phe Phe Lys Trp Trp Ser Leu His Phe Leu Pro Lys Leu Glu Val
670 675 680
Leu Asp Leu Ala Gly Asn Gln Leu Lys Ala Leu Thr Asn Gly Ser Leu
685 690 695
Pro Ala Gly Thr Arg Leu Arg Arg Leu Asp Val Ser Cys Asn Ser Ile
700 705 710
Ser Phe Val Ala Pro Gly Phe Phe Ser Lys Ala Lys Glu Leu Arg Glu
715 720 725
Leu Asn Leu Ser Ala Asn Ala Leu Lys Thr Val Asp His Ser Trp Phe
730 735 740 745
Gly Pro Leu Ala Ser Ala Leu Gln Ile Leu Asp Val Ser Ala Asn Pro
750 755 760
Leu His Cys Ala Cys Gly Ala Ala Phe Met Asp Phe Leu Leu Glu Val
765 770 775
Gln Ala Ala Val Pro Gly Leu Pro Ser Arg Val Lys Cys Gly Ser Pro
780 785 790
Gly Gln Leu Gln Gly Leu Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys
795 800 805
Leu Asp Glu Ala Leu Ser Trp Asp Cys Phe Ala Leu Ser Leu Leu Ala
810 815 820 825
Val Ala Leu Gly Leu Gly Val Pro Met Leu His His Leu Cys Gly Trp
830 835 840
Asp Leu Trp Tyr Cys Phe His Leu Cys Leu Ala Trp Leu Pro Trp Arg
845 850 855
Gly Arg Gln Ser Gly Arg Asp Glu Asp Ala Leu Pro Tyr Asp Ala Phe
860 865 870
Val Val Phe Asp Lys Thr Gln Ser Ala Val Ala Asp Trp Val Tyr Asn

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    875                 880                 885
Glu Leu Arg Gly Gln Leu Glu Glu Cys Arg Gly Arg Trp Ala Leu Arg
890                 895                 900                 905
Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro Gly Lys Thr Leu Phe Glu
                910                 915                 920
Asn Leu Trp Ala Ser Val Tyr Gly Ser Arg Lys Thr Leu Phe Val Leu
            925                 930                 935
Ala His Thr Asp Arg Val Ser Gly Leu Leu Arg Ala Ser Phe Leu Leu
        940                 945                 950
Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys Asp Val Val Val Leu Val
    955                 960                 965
Ile Leu Ser Pro Asp Gly Arg Arg Ser Arg Tyr Val Arg Leu Arg Gln
970                 975                 980                 985
Arg Leu Cys Arg Gln Ser Val Leu Leu Trp Pro His Gln Pro Ser Gly
                990                 995                1000
Gln Arg Ser Phe Trp Ala Gln Leu Gly Met Ala Leu Thr Arg Asp Asn
           1005                1010                1015
His His Phe Tyr Asn Arg Asn Phe Cys Gln Gly Pro Thr Ala Glu
1020                1025                1030
```

1020 1025 1030 1032

CLAIMS

1. An isolated Toll-like-receptor polypeptide consisting essentially of
   (i) the amino acid sequence SEQ ID NO: 2;
   (ii) a variant thereof which has immunomodulatory activity; or
   (iii) a fragment of (i) or (ii) which has immunomodulatory activity.

2. A polypeptide according to claim 1 wherein the variant (ii) has at least 70% identity to the amino acid sequence of SEQ ID NO: 2.

3. A polypeptide according to claim 2 which has at least 70% identity to the amino acid sequence of SEQ ID NO: 2 over a region of at least 70% of the full-length sequence provided by SEQ ID No.1 and exhibits toll-like receptor functionality.

4. A polypeptide according to claim 1 or claim 2 wherein the variant (ii) has at least 95% identity to the amino acid sequence of SEQ ID NO: 2.

5. A polypeptide according to claim 2 which has at least 95% identity to the amino acid sequence of SEQ ID NO: 2 over a region of at least 60 contiguous amino acids and exhibits toll-like receptor functionality.

6. A polypeptide according to claim 1 wherein the fragment (iii) is a peptide of up to 150 amino acids in length and exhibits toll-like receptor functionality.

7. A polynucleotide encoding a polypeptide according to any one of claims 1-3.

8. A polynucleotide encoding a Toll-like receptor polypeptide which has immunomodulatory activity, which polynucleotide consists essentially of:
   (a) the nucleic acid sequence of SEQ ID NO: 1;
   (b) a sequence complementary thereto;

(c) a sequence which hybridises under stringent conditions to a sequence as defined in (a) or (b);

(d) a sequence that is degenerate as a result of the genetic code to a sequence as defined in (a), (b) or (c); or

(e) a sequence having at least 60% identity to a sequence as defined in (a), (b), (c) or (d).

9. A polynucleotide according to claim 7 or claim 8 which is mRNA.

10. A polynucleotide according to claim 7 or claim 8 which is DNA.

11. A polynucleotide according to claim 7 or claim 8 which is cDNA.

12. An isolated toll-like receptor polypeptide which is obtainable by expression *in vivo* or *in vivo* of a polynucleotide according to claim 7 or claim 8.

13. A polypeptide according to claim 12 which has the structural features conserved amongst toll-like receptors.

14. An expression vector comprising a polynucleotide sequence according to any one of claims 7 to 11, which is capable of expressing a polypeptide according to any one of claims 1 to 3 or claim 12.

15. An expression vector according to claim 14 which is a plasmid, phage or viral vector.

16. A host cell comprising an expression vector according to claim 14 or claim 15.

17. A polyclonal or monoclonal antibody, or a chimera or fragment thereof, which is specific for a polypeptide according to any one of claims 1 to 3.

18. A method for identification of a compound that modulates Toll-like receptor activity, which method comprises contacting a polypeptide according to any one of claims 1 to 3 or claim 12 with a test substance and monitoring for immunomodulatory activity.

19. A compound which modulates Toll-like receptor activity and which is identifiable by a method according to claim 18.

20. A compound according to claim 19 which is a peptide or polypeptide.

21. A compound according to claim 19 which is an oligonucleotide or polynucleotide.

22. A method of treating a subject having an inflammatory or cardiovascular disorder, systemic infection or autoimmune disease that is responsive to Toll-like receptor modulation, which method comprises administering to said subject an effective amount of a compound according to any one of claims 19 to 21 or an antibody according to claim 17.

23. A method according to claim 22 wherein the disorder is a viral, fungal or bacterial infection, asthma, rhinitis, chronic obstructive pulmonary disease (COPD), emphysema, an inflammatory bowel disease such as ulcerative colitis or Crohn's disease, rheumatoid arthritis, osteoarthritis, psoriasis, Alzheimers disease, atherosclerosis, Multiple Sclerosis, diabetes or septic shock syndrome associated with systemic infection involving gram positive or gram negative bacteria. .

24. A polypeptide comprising a fragment or variant of SEQ ID NO: 2, which is capable of inhibiting the activity of TLR9 having the amino acid sequence of SEQ ID NO: 2 or a functional variant thereof, for use in the treatment of an immune or inflammatory disorder.

25. A polypeptide according to any one of claims 1 to 3 or claim 12, a polynucleotide according to claim 7 or claim 8 or a compound according to claim 19 for use as an adjuvant.

26. The use of a compound according to claim 19 in the manufacture of a medicament for the treatment of an immune or inflammatory disorder.

27. The use of a polypeptide comprising a fragment or variant of SEQ ID NO: 2, which is capable of inhibiting the activity of TLR9 having the amino acid sequence of SEQ ID NO: 2 or a functional variant thereof, in the manufacture of a medicament for the treatment of an immune or inflammatory disorder.

28. The use of a polypeptide according to any one of claims 1 to 3 or claim 12, a polynucleotide according to claim 7 or claim 8 or a compound according to claim 19 in the manufacture of an adjuvant or vaccine formulation.

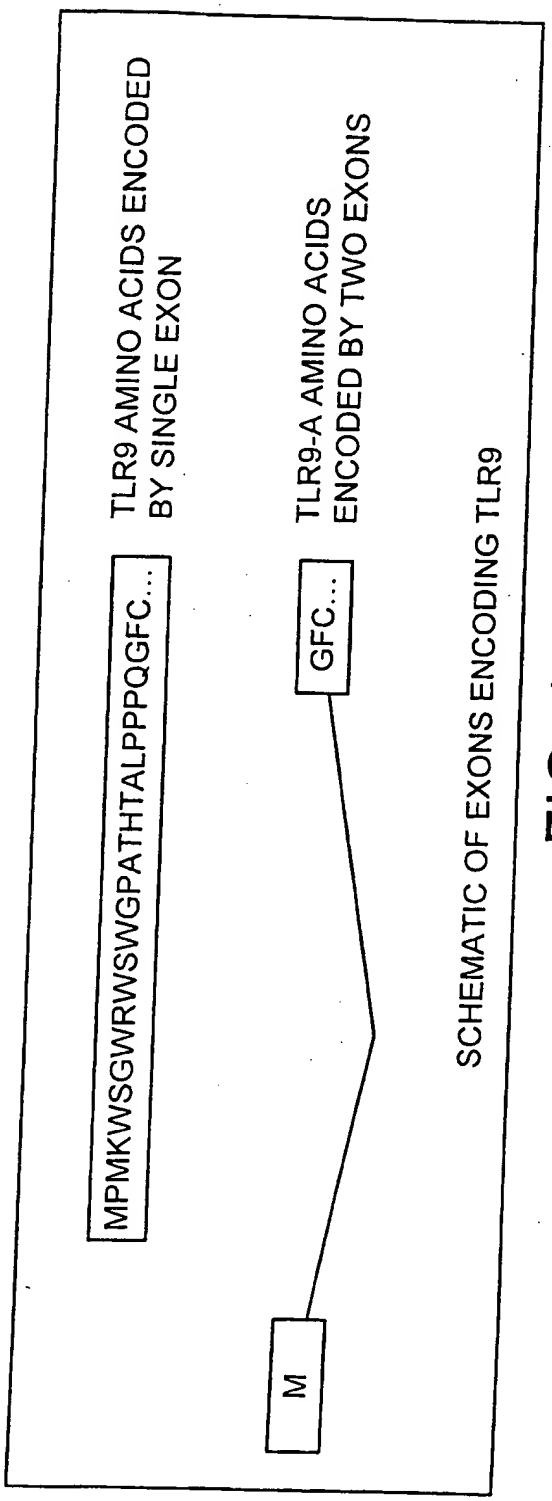
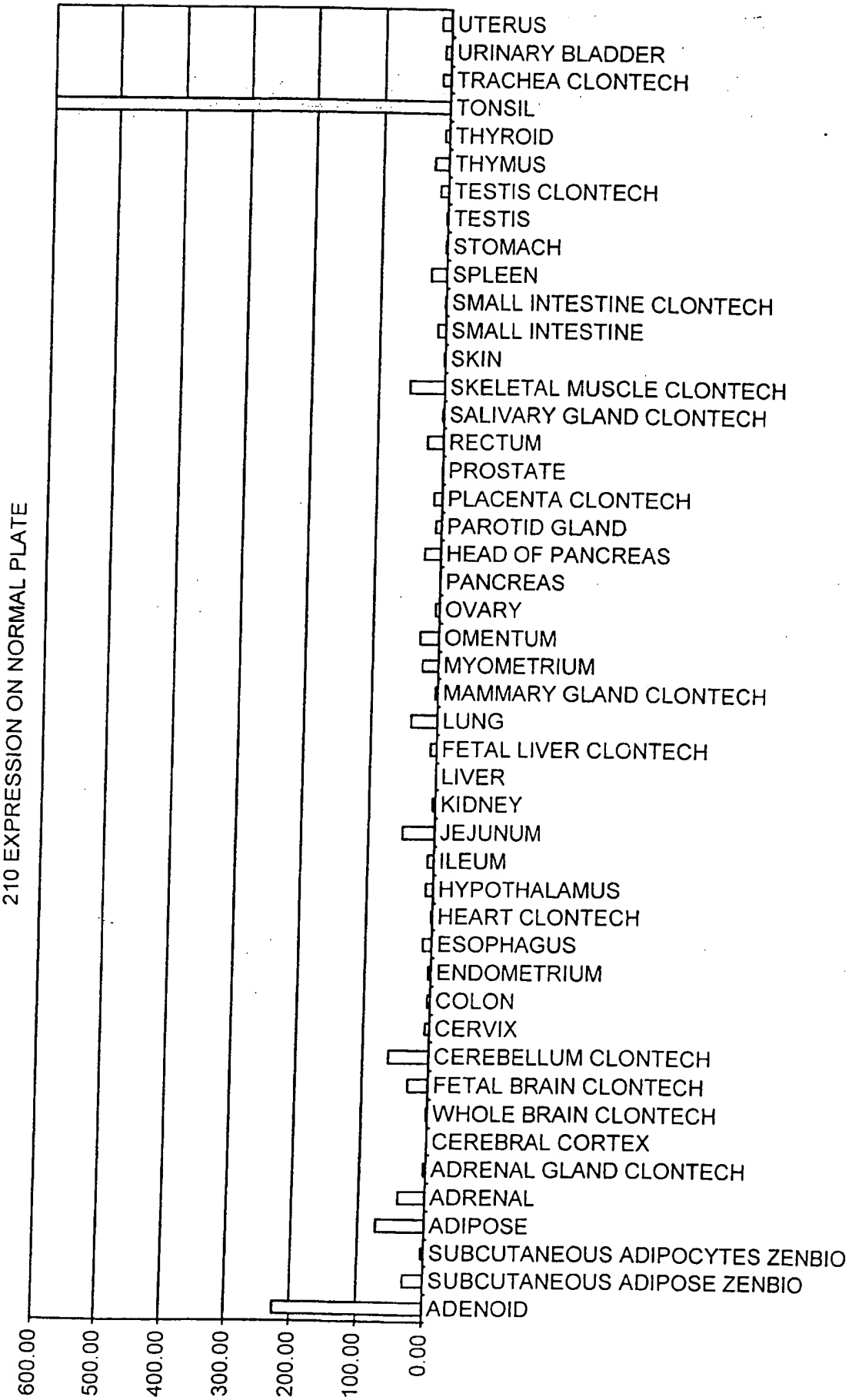

SCHEMATIC OF EXONS ENCODING TLR9
TLR9 AMINO ACIDS ENCODED BY SINGLE EXON
TLR9-A AMINO ACIDS ENCODED BY TWO EXONS
MPMKWSGWRWSWGPATHTALPPPQGFC....
GFC....
M

FIG. 1

FIG. 2


210 EXPRESSION ON NORMAL PLATE
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300.00
200.00
100.00
0.00
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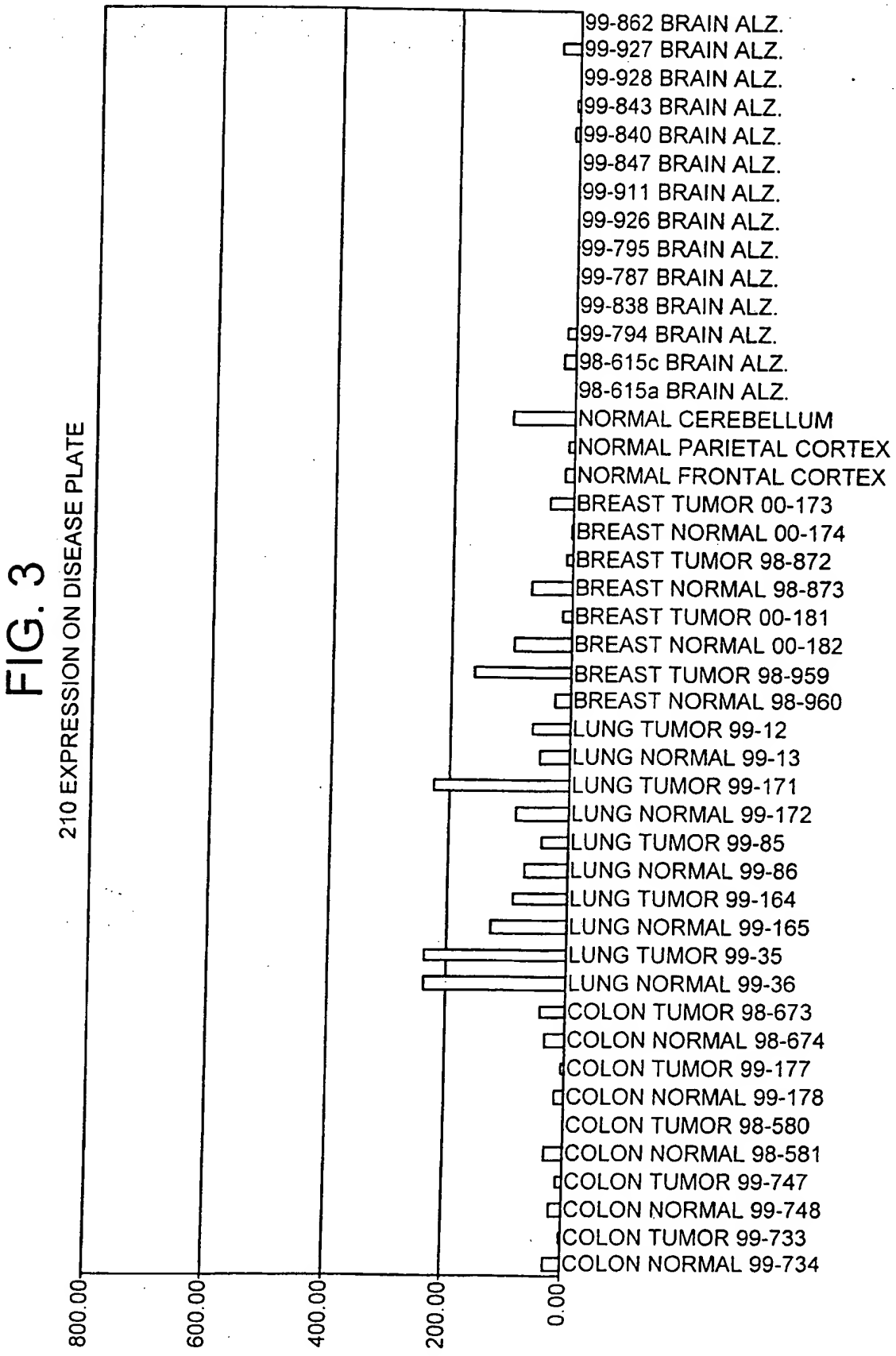
TRACHEA CLONTECH
TONSIL
THYROID
THYMUS
TESTIS CLONTECH
TESTIS
STOMACH
SPLEEN
SMALL INTESTINE CLONTECH
SMALL INTESTINE
SKIN
SKELETAL MUSCLE CLONTECH
SALIVARY GLAND CLONTECH
RECTUM
PROSTATE
PLACENTA CLONTECH
PAROTID GLAND
HEAD OF PANCREAS
PANCREAS
OVARY
OMENTUM
MYOMETRIUM
MAMMARY GLAND CLONTECH
LUNG
FETAL LIVER CLONTECH
LIVER
KIDNEY
JEJUNUM
ILEUM
HYPOTHALAMUS
HEART CLONTECH
ESOPHAGUS
ENDOMETRIUM
COLON
CERVIX
CEREBELLUM CLONTECH
FETAL BRAIN CLONTECH
WHOLE BRAIN CLONTECH
CEREBRAL CORTEX
ADRENAL GLAND CLONTECH
ADRENAL
ADIPOSE
SUBCUTANEOUS ADIPOCYTES ZENBIO
SUBCUTANEOUS ADIPOSE ZENBIO
ADENOID

FIG. 3


210 EXPRESSION ON DISEASE PLATE
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99-927 BRAIN ALZ.
99-928 BRAIN ALZ.
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99-840 BRAIN ALZ.
99-847 BRAIN ALZ.
99-911 BRAIN ALZ.
99-926 BRAIN ALZ.
99-795 BRAIN ALZ.
99-787 BRAIN ALZ.
99-838 BRAIN ALZ.
99-794 BRAIN ALZ.
98-615c BRAIN ALZ.
98-615a BRAIN ALZ.
NORMAL CEREBELLUM
NORMAL PARIETAL CORTEX
NORMAL FRONTAL CORTEX
BREAST TUMOR 00-173
BREAST NORMAL 00-174
BREAST TUMOR 98-872
BREAST NORMAL 98-873
BREAST TUMOR 00-181
BREAST NORMAL 00-182
BREAST TUMOR 98-959
BREAST NORMAL 98-960
LUNG TUMOR 99-12
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COLON TUMOR 99-733
COLON NORMAL 99-734

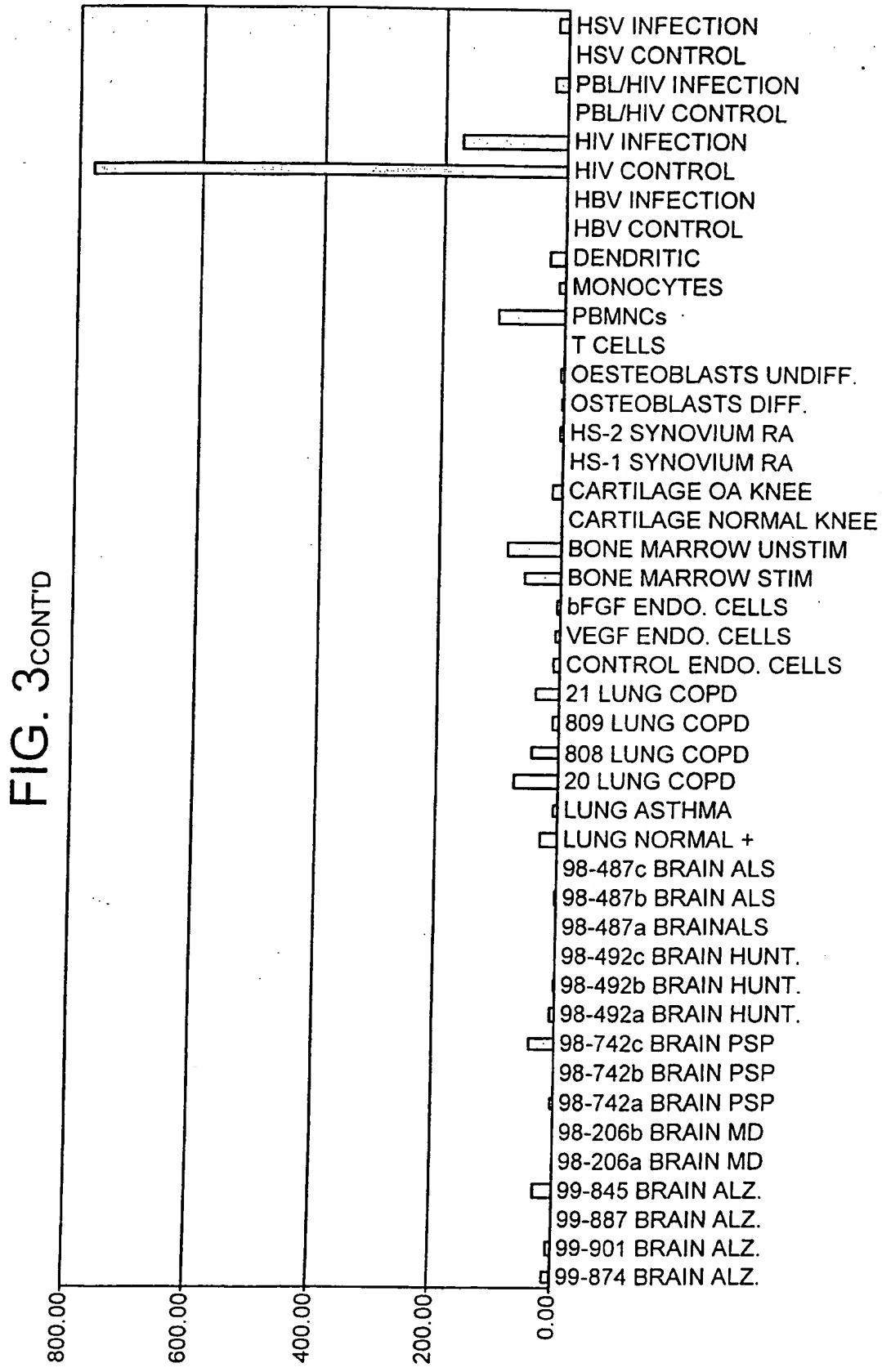
HSV INFECTION
HSV CONTROL
PBL/HIV INFECTION
PBL/HIV CONTROL
HIV INFECTION
HIV CONTROL
HBV INFECTION
HBV CONTROL
DENDRITIC
MONOCYTES
PBMNCs
T CELLS
OESTEOBLASTS UNDIFF.
OSTEOBLASTS DIFF.
HS-2 SYNOVIUM RA
HS-1 SYNOVIUM RA
CARTILAGE OA KNEE
CARTILAGE NORMAL KNEE
BONE MARROW UNSTIM
BONE MARROW STIM
bFGF ENDO. CELLS
VEGF ENDO. CELLS
CONTROL ENDO. CELLS
21 LUNG COPD
809 LUNG COPD
808 LUNG COPD
20 LUNG COPD
LUNG ASTHMA
LUNG NORMAL +
98-487c BRAIN ALS
98-487b BRAIN ALS
98-487a BRAINALS
98-492c BRAIN HUNT.
98-492b BRAIN HUNT.
98-492a BRAIN HUNT.
98-742c BRAIN PSP
98-742b BRAIN PSP
98-742a BRAIN PSP
98-206b BRAIN MD
98-206a BRAIN MD
99-845 BRAIN ALZ.
99-887 BRAIN ALZ.
99-901 BRAIN ALZ.
99-874 BRAIN ALZ.
800.00
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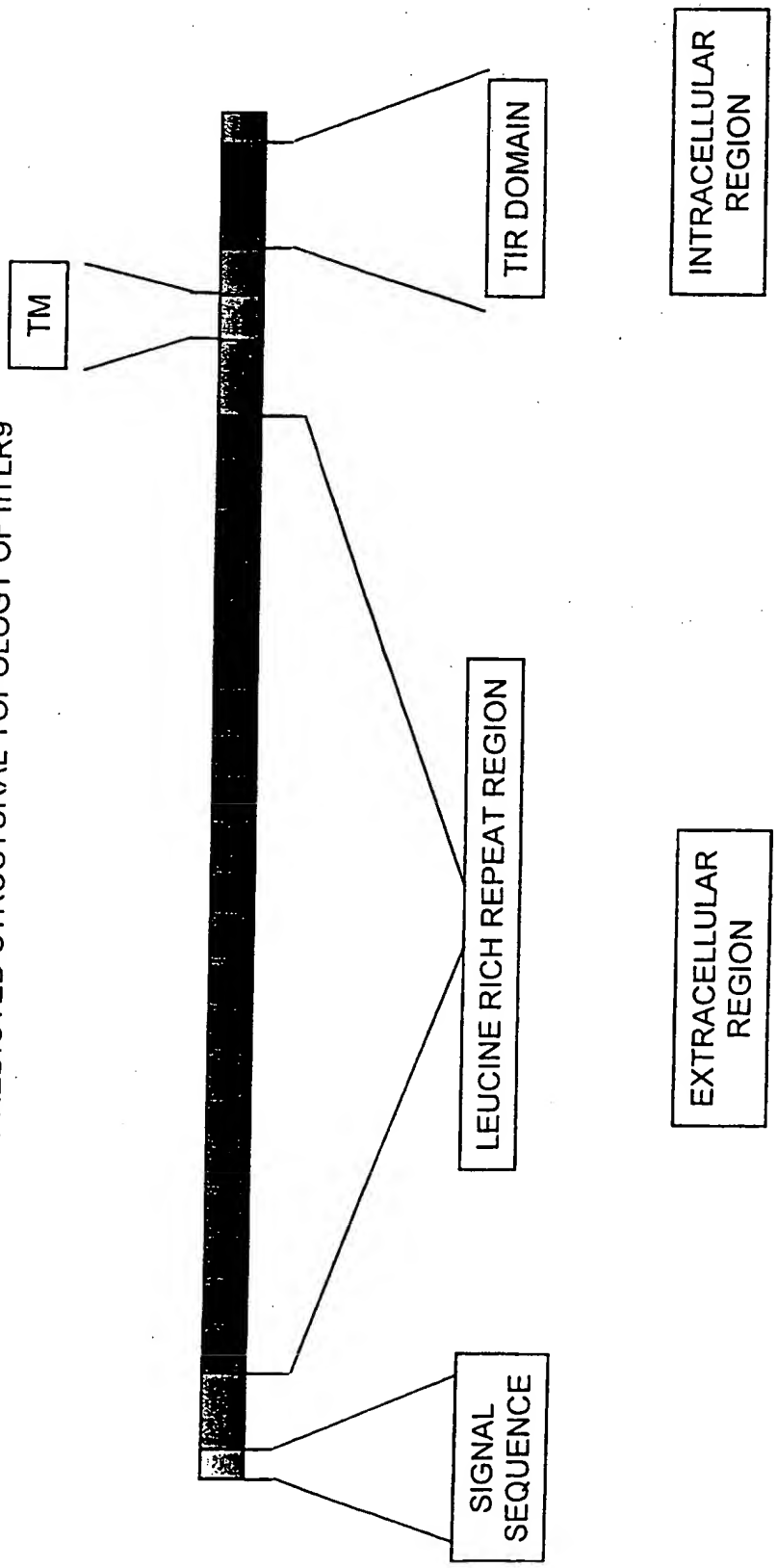
FIG. 3CONT'D

FIG. 4

PREDICTED STRUCTURAL TOPOLOGY OF hTLR9



INTERNATIONAL SEARCH REPORT

Intern. ɔnal Application No
PCT/GB 01/00299

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/63 C12N5/10 G01N33/68 C07K14/705
C07K16/28 A61K31/7088 A61K38/17 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBL, STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 50547 A (SCHERING CORP) 12 November 1998 (1998-11-12) 99.8% identity in 1008 bp overlap between SEQ ID NO 33 of WO9850547 and SEQ ID NO 1 99.7% identity in 336 amino acids overlap between SEQ ID NO 34 of WO9850547 and SEQ ID NO 2 page 56, line 23 -page 60, line 14; claims 1-16; examples 1-11 --- -/--	1-18, 22-25, 27,28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2001	11/07/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Devijver, K

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter .onal Application No
PCT/GB 01/00299

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! accession: AC006252, 29 December 1998 (1998-12-29) MUZNY D ET AL: "Homo sapiens 3p21.1 contig 9 PAC RPCI5-1157M23 (Roswell Park Cancer Institute Human PAC Library) complete sequence. " XP002170407 100% identity in 3165 bp overlap (between positions 26803-29967) with SEQ ID NO 1 100% identity in 1055 amino acids overlap (between positions 26803-29967) with SEQ ID NO 2 (tfasta) ---	7-11
P,X	HEMMI HIROAKI ET AL: "A Toll-like receptor recognizes bacterial DNA" NATURE,MACMILLAN JOURNALS LTD. LONDON,GB, vol. 408, no. 6813, 7 December 2000 (2000-12-07), pages 740-745, XP002168474 ISSN: 0028-0836 cited in the application the whole document -& DATABASE EMBL 'Online! accession: AB045180, 13 December 2000 (2000-12-13) AKIRA S ET AL: "Homo sapiens TLR9 mRNA for toll-like receptor 9, complete cds." XP002170408 99.7% identity in 3113 bp overlap with SEQ ID NO 1 100% identity in 1031 amino acids overlap with SEQ ID NO 2 -----	1-18, 22-25, 27,28

3

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 19-21,26; in part: 22,23,25,28

Claim 19 refers to a compound which modulates Toll-like receptor activity without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claim is ambiguous and vague, and its subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).
No meaningful search can be carried out for such a purely speculative claim whose wording is, in fact, a mere recitation of the result to be achieved.
The above comment also applies for claims 20,21 and 26; and in part for claims 22,23,25 and 28.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna...ial Application No
PCT/GB 01/00299

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9850547 A	12-11-1998	AU 7175498 A	27-11-1998
		BR 9808747 A	11-07-2000
		CN 1263555 T	16-08-2000
		EP 0980429 A	23-02-2000
		NO 995458 A	08-11-1999
		PL 336635 A	03-07-2000
		SK 146599 A	11-07-2000
		HU 0001462 A	28-07-2000

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/10408

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG (Biosis, Embase, Medline, Cancerlit, Scisearch, Derwint)
searcg terms: CpG(w)S, CpG(w)N, CpG, immunostimul?, site(w)directed(w)mutagenesis, oligodeoxynucleotides, ODN, antisense